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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR2579 for a patent by INTREAT PTY LIMITED as filed on 17 January 2001.

I further certify that pursuant to the provisions of Section 38(1) of the Patents Act 1990 a complete specification was filed on 17 January 2002 and it is an associated application to Provisional Application No. PR2579 and has been allocated No. 2002224664.

WITNESS my hand this
Twelfth day of March 2007

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AUSTRALIA
Patents Act 1990
SPECIFICATION FOR A PROVISIONAL PATENT APPLICATION

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Invention Title: **Diagnosis And Treatment Of Malignant Lymphoma**

The following statement is a description of this invention

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This invention relates to diagnosis and/or treatment of malignant lymphoma. The invention is particularly concerned with malignant lymphoma in humans but is not necessarily limited thereto.

Part of the basis for the invention is found in research into purinergic receptor subtypes (P2X) in lymphocytes. It is known that P2X binding sites are present in lymphocytes and it has been possible to detect the distribution of the P2X receptors using antibodies specific to each of the subtypes. Subtypes $P2X_1$ to $P2X_7$ have been identified. The $P2X_7$ subtype has been implicated in apoptosis or programmed cell death in many cell types. It is referred to as a cytolytic receptor capable of forming pores that enable the cell to be flooded with excess calcium rather than simply acting as a calcium channel. The B-cells in patients with malignant lymphoma express $P2X_7$ receptors that are unable to form pores. These are termed non-functional receptors. The invention uses a $P2X_7$ subtype-specific antibody to specifically detect non-functional $P2X_7$ receptors expressed on B-cells.

It has now been found that, in patients with malignant lymphoma, the non-functional $P2X_7$ receptors can be detected by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors so that the cause of the conformational change in the receptors relates to interaction of the receptors with two bound molecules of adenosine triphosphate (ATP). The ATP molecules act as receptor agonists, so that when bound, they open a channel through the cell membrane for the flow of calcium ions. If ATP binding is disrupted, the conformation of the receptors is altered and can be detected using an antibody specially designed to detect the change adjacent to the ATP binding site. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors.

ATP can induce cytolysis in leukocytes including lymphocytes, thymocytes, macrophages and dendritic cells through the $P2X_7$ receptors expressed on the cell surface. Lymphoma develops from malignant clones that escape cytolytic destruction. This process leads to the progressive accumulation of malignant B-lymphocytes and thus lymphadenopathy and/or splenomegaly. $P2X_7$ receptors open channels through the cell membrane within a second and this is followed by the formation of a pore within a few tens of seconds with a continued supply of ATP that induces apoptosis. Channel opening of $P2X_7$ receptors on leukocytes is terminated through the rapid hydrolysis of ATP agonist by ecto-ATPases and ecto-

ATPdiphosphohydrolase (ecto-ATPDase). These enzymes regulate numerous physiological processes that are dependent on ATP. Substrate specificity of ATPase and ATPDase activity on lymphocytes indicates the presence on the lymphocytes of more than one type on the cell surface, including CD39. Proliferation of one or more of these ATPases or ATPDases could limit the supply of ATP needed to control $P2X_7$ pore formation and the subsequent programmed cell death needed to regulate B-cell numbers.

Because current studies and investigations may not fully explain the working of the invention, it is necessary to define the invention in a number of aspects, as set out below. It is possible and likely that there will be overlap of at least some of those aspects.

Accordingly, in a first aspect, the invention provides an antibody for detection of malignant lymphoma, the antibody being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors. The antibody may be either polyclonal or monoclonal and is preferably directed against an epitope located in the extracellular domain adjacent to the ATP binding sites and incorporating the proline at amino acid 210 in the human $P2X_7$ sequence that undergoes cis/trans isomerisation, with the cis conformer associated with the non-functional conformation. It is apparent that the embodiment of the invention covers alternative sequences that similarly distinguish functional and non-functional receptors through detection of the conformational changes occurring when ATP binds so the change detected may be in an amino acid other than the proline referred to above, or in some other respect.

In a second aspect, the invention provides a pharmaceutical composition for treatment or prevention of malignant lymphoma in a patient, the composition including a pharmaceutically effective amount of one or more substances adapted to regulate the expression of ATPases (enzymes) that control the supply of ATP to $P2X_7$ receptors in the B-cells of the patient. The ATPases control the local supply of ATP to the $P2X_7$ receptors so as to reduce the concentration of ATP available for binding to the $P2X_7$ receptors and so deactivate them leading to a significant reduction in programmed B-cell death. These ATPases may be specifically expressed on the surface of the B-cells and appear to be up-regulated in malignant lymphoma. Preferably, application of a specific ATPase inhibitor may be used to regulate the availability of ATP on the $P2X_7$ receptors, so regulating programmed B-cell death.

In a third aspect, the invention provides a pharmaceutical composition for treatment or prevention of malignant lymphoma in a patient, the composition including a pharmaceutically effective amount of one or more substances adapted to reduce the ability of ATPases to deplete the local supply of ATP to $P2X_7$ receptors in the B-cells of the patient. This could take the form of ATP analogues that are non-hydrolysable by the ATPases but are still able to bind to the $P2X_7$ receptors and activate them. This approach has the added advantage of removing L-selectin from B-cells so that they are more easily removed from lymph nodes enabling them to be released into the circulation where they will be more accessible to treatments.

The invention also provides a method of treating or preventing malignant lymphoma in a patient, comprising administering to the patient a pharmaceutical composition as defined in any of the aspects above.

The invention also provides the use of a pharmaceutical composition defined in any of the aspects above, in the treatment or prevention of malignant lymphoma in a patient.

Clinical trials to be carried out shortly should reveal examples of substances suitable for use in the compositions of the inventions. At this stage, it is anticipated that non-hydrolysable ATP analogues or di-adenosine polyphosphates may be useful while a search is made to find suitable ATPase inhibitors for treatment. The diagnostic can be used in standard microscopy, confocal microscopy or fluorescence activated cell sorting as well as normal immunohistochemical techniques of lymph biopsies. Further, the pattern of use of one or more of the above pharmaceutically effective agents may need to be altered for optimum effect.

It is contemplated that the control of supply of ATP to the P2X receptors may be capable of affecting many diseases or conditions. Accordingly, this invention provides, in a broad aspect, a pharmaceutical composition for treatment or prevention of a disease or medical condition in a patient, the composition including a pharmaceutically effective amount of one or more substances adapted to control supply of ATP to P2X receptors.

The supply may be controlled in any effective way. Some examples have already been discussed above, such as the regulation of ATPases.

The invention also provides a method of treating or preventing disease or medical conditions in a patient, comprising administering to the patient a pharmaceutical composition as defined in the broad aspect above.

The invention also provides the use of a pharmaceutical composition defined in the broad aspect above, in the treatment or prevention of diseases or medical conditions in a patient.

It will be apparent to those skilled in the art that many obvious modifications and variations may be made to the embodiments described herein without departing from the spirit or the scope of the invention.

Dated this 17th day of January, 2001

Intreat Pty Limited

by its Patent Attorneys

Chrysiliou Law